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THE DEVELOPMENT AND STRUCTURE OF THE LARVA OF PARAGORDIUS.

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The embryology of the *Gordiacea* is a subject full of mystery and interest, notwithstanding the attempts at its solution. It is one fraught with technical difficulties, and one where the obtaining of material is usually a matter of chance. With a peculiar delight then, after many vain attempts to collect the eggs, I found in March and April of the present year numerous adults of both sexes, with egg-strings in abundance, of *Paragordius varius* (Leidy) in a small stream that passes through the town of Austin, Texas.

Ovipositing females were brought to the laboratory, and the egg-strings kept for weeks in small jars of water aerated by plants. The full-formed larval stage is reached in from ten to twelve days, and these larvæ live for more than a week longer before they leave the egg-membranes. After leaving the latter they live for only a few days unless they reach their proper host.

In Texas, as in Pennsylvania, the last host is the large cricket (*Acheta abbreviata*); in this host are found individuals from about one-third the ultimate size to the adult stage. As is well known for all the species of *Gordiacea*, the adults leave their terminal hosts and come into the water, where the eggs are fertilized and laid. Whether the cricket is the only host of *Paragordius varius*, or whether there is a preceding one into which the larva first enters, I have not yet been able to determine. Attempts were made to infect crickets with larvæ, by crushing the egg-strings containing the latter so as to liberate the larvæ, and placing drops of water filled with the larvæ upon grass fed to the crickets. None of the adult crickets lived longer than a month in confinement; and each one out of about 75 kept under observation was examined in vain at the time of its death for *Paragordius* larvæ. Crushed egg-strings were placed in water containing Amphipods (Gammarids); at the end of two weeks the Amphipods showed numerous larvæ encysted in the intestine and musculature, but these larvæ underwent no further development. Terrestrial Isopods (*Oniscus*) found in damp places were placed in contact with water containing larvæ, and such water was dropped upon their mouths; two weeks later a single one of them showed a single larva in the intestine, encysted but dead. Pieces of egg-strings were placed in aquaria with brook minnows; the fish some three weeks later showed larvæ encysted in the intestine, and a few in the muscles, but here again no further development of the larvæ was obtained. Finally infection attempts were made with tadpoles of *Bufo.* The first lot of tadpoles were small (but operculum present), and crushed egg-strings were placed in their water on April 12; most of the tadpoles died between the 16th and the 18th of April, the last on the 19th; the autopsy showed large numbers of larvæ (not encysted) in the intestine, but most if not all of the larvæ were dead. About the same results were reached with two other lots. Larvæ placed in water containing mosquito larvæ (*Culex*) encysted themselves in the mosquitoes and soon caused their death.

These results are, of course, quite indecisive, except in showing that mosquito larvæ and toad tadpoles cannot be normal hosts of the parasites. So I have not been able to secure the early post-larval development of *Paragordius,* and in the present contribution shall deal with that literature only which concerns the development up to the larval stage.

Since the ultimate host is a land insect, while the parasites are at first aquatic, it is of value to determine how long the mature worms can withstand desiccation. One adult female within a few hours after emerging from a cricket was placed in a dry dish; four hours afterward she was alive, but with dry cuticle; eighteen hours afterward she was shriveled up and did not resuscitate on being placed in water. Several adult females, a few hours after escaping from crickets, were placed in an open dish on moist filter paper; twenty-four hours afterward they were still alive though the body surface was dry, and on placing them in water they lived for several days. Egg-strings when dried shrivel up and the eggs die quickly.

Portions of egg-strings at timed periods were preserved in three fixatives: Zenker's fluid, made up of 5 per cent. of corrosive sublimate and 5 per cent. of glacial acetic acid in Müller's solution (bichromate of potash 2 parts, sulphate of soda 1 part, water 100 parts); a mixture of 95 per cent. alcohol, 3 per cent. nitric acid, and aqueous solution of corrosive sublimate in equal parts; and a mixture of glacial acetic acid 10 parts, and 95 per cent. alcohol 90 parts. The fixation with Zenker's fluid is by far the best of these, producing no distortion and preserving admirably delicate cellular details. To my surprise I found, contrary to the results of other workers, that the ova, despite their membranes, are readily penetrated, and that all alcoholic and strong acid solutions are to be avoided. Flemming's fluid (osmic acid, acetic acid and chromic acid) penetrates easily, but blackens the tissues too quickly. A fixation of three to twenty hours in Zenker's fluid is followed by rinsing in distilled water for fully the same length of time; then the egg-strings are brought through successive alcohol grades up to 83 per cent. They remain in the latter for several days, then are brought down gradually to distilled water. With each egg-string in a narrow, round-bottomed vial, I take a glass rod and thoroughly crush the egg-string in water after it has been well hardened in alcohol; thus the eggs are easily freed from each other and none are lost. An aqueous stain is then poured upon them; and for material fixed in Zenker's fluid I found Delafield's hæmatoxyline, diluted with an equal volume of water, for two to three hours, the best. The ova, still within the same vial, are brought up through successive grades of alcohol to absolute alcohol, half an hour in each grade; then into a mixture of equal parts of absolute alcohol and xylol; then into pure xylol. They are then mounted in Canada balsam. These methods have been given in detail, because only after numerous failures have I been able to secure fine preparations. In the structure of the larva much can be determined by study of it in life. And one can best investigate the armature of the proboscis by causing it to be permanently evaginated through treatment with a rather strong aqueous solution of caustic potash; this swells up the whole body. Sections were made of numerous egg-strings, but the paraffine imbedding causes great distortion of the cells, and it is necessary to imbed for several hours. The whole mounts of the ova and larvæ were the most instructive.

1. The Early Development.

The structure of the adult reproductive organs has been previously described by me (1903) in detail, and it is necessary here to recall only a few points to make the development clear. The ovaries are a pair of long tubes extending nearly the entire length of the body, each consisting of a longitudinal tube, the "uterus" of the authors, and of very numerous lateral diverticula or pouches, "ovaries" in the narrow sense, each of which communicates by an aperture with the uterus. The posterior ends of the two uteri are ciliated, and are termed the oviducts, and these open into the atrium, into the anterior end of which opens also the large receptaculum seminis. The end of the intestine communicates with the atrium, and from that point to the posterior end of the body extends the tubular cloaca, with the cloacal aperture between the three tail lobes. The atrium is lined with a glandular epithelium, as is also the proximal end of the cloaca; the remainder of the cloaca bears a cuticular lining.

The egg-cells contained in the lateral diverticula of the ovaries of adult females (individuals whose external cuticula is of a dark color) are all ovocytes of the first order at the end of the growth period (fig. 1, Pl. XLIX). The nucleus is more or less central in position, contains one large deeply staining nucleolus, and a nuclear sap which shows no trace of chromatin and only in a few cases exceedingly delicate linin fibres; even strong iron hæmatoxyline stains fail to demonstrate chromatin particles. The cell-body evinces a zone of yolk globules (shaded in figs. 1 and 15), placed near the periphery; while the cytoplasm is coarsely alveolar, and the meshes of very regular size in the perinuclear area.

While these ovocytes are passing from the "ovaries" to the "uteri," but before they have entered the latter, their nuclei are in prophases of the first maturation mitosis; such stages are shown in figs. 2–5. The nucleolus becomes smaller and stains less intensely, while there appear, usually close to it, chromosomes in the form of long loops (figs. 2, 3); these do not first appear simultaneously, but successively. These chromosomes shorten into the form of minute dumbbell-shaped bodies, seven in number (fig. 4), and for the first time is seen in the nucleus an achromatic network (fig. 5). These are bivalent chromosomes, as shown by their shape and by the fact that there are fourteen single chromosomes in the cells of the embryo. Two points are remarkable in regard to them: first, that no trace of them is to be seen in the preceding rest stage; and second, that they appear in succession close to the nucleolus, and later are frequently found in a compact group—a condition infrequent in a prophase. The evidence is that these chromosomes come out of the nucleolus, and that during the rest stage they are contained within it. Such a condition has recently been demonstrated for the egg of *Asterias* by Hartmann (1902); but that it is by no means a usual condition in Metazoa, on the contrary is decidedly unusual, was previously shown by me (1898). As the ovocyte enters the uterus the seven double chromosomes are arranged in the equator of the first polar spindle (fig. 6; fig. 7, a pole view of the spindle); this has the form of the corresponding spindle of *Ascaris*, and no centrosomes are demonstrable. As the ovocytes, closely compacted together, are propelled rapidly backward along the uteri and so through the oviducts into the atrium, the spindle moves gradually nearer the periphery of the egg.

The females are impregnated with sperm, and the receptaculum seminis filled with a great mass of it, before the ovocytes enter the uteri, so before the first polar spindle is formed. This I have proved by examination of numerous adult females. There is an intimate coition, the male placing his cloacal aperture against that of the female; and the sperm has to traverse the length of the cloaca before reaching the receptaculum. Ova will apparently not develop unless fertilized, for there was no development at all in the egg-string of a female whose receptaculum was without spermatozoa. In the testes of the adult male only mature spermatozoa are present, and in this species there appears to be no spermatophores.

When the ovocytes reach the atrium they become surrounded by the minute flagellate spermatozoa issuing from the receptaculum, and in the posterior part of the atrium and the proximal end of the cloaca the sperm enters the egg (fig. 6). The pole spindle is now excentric; the spermatozoon enters usually at a point of the periphery furthest removed from the spindle, but sometimes quite near it (fig. 6). On strongly destained hæmatoxyline preparations the spermatozoon head is sharply distinguishable. The ovocytes are propelled backward within the cloaca compacted into an egg-string. While the ovarian egg has a limiting membrane of very delicate nature, the egg in the proximal part of the cloaca has a thicker membrane that stains with hæmatoxyline (fig. 6); this increases in thickness as the egg progresses backward and becomes the outermost envelope of the egg (figs. 8, 10). At the posterior end of the cloaca each egg shows a spermatozoon within the cell membrane, and the first polar spindle at the periphery of the cell (fig. 8). At this stage the egg has two membranes: the outer, thinner one staining with chromatin stains, already mentioned, and a thicker inner one that stains faintly with cytoplasmic stains (fig. 8); both are closely adherent to the cytoplasm. But where the polar spindle touches the periphery these membranes are not present. It is probable that both these membranes are products of the cytoplasm, and not of any gland cells of the genital passages (as I had previously opined), else one could not explain their absence in the region of the polar spindle. The outer membrane has at its inception probably a glutinous nature, serving to hold the eggs together in a string.

The eggs pass out of the cloaca in a cylindrical continuous string, usually much convoluted, and in the first few days snowy white; the worm may occupy as much as twenty-four hours in the discharge of its egg-string, and then, with its body flaccid and flattened, it expires.

The first pole-body is cut off by the egg shortly after the expulsion from the body, and when it is cut off the two cell-membranes separate it from the surface of the egg (fig. 9). Shortly afterward these two membranes swell, probably by osmotic action of the surrounding water, and together compose a double membrane removed from the surface of the egg (fig. 10), while the cytoplasm has formed a third delicate membrane which remains adherent to it. By this means the first polar body becomes removed from the surface of the egg. The ovocyte of the second order (fig. 10) shows the second polar spindle, like the first in form, and without an intervening rest stage, and this also contains seven chromosomes (fig. 11). The chromosomes are too minute to allow a determination of which of these is the reduction division; the second polar body is then cut off (fig. 12), but remains adherent to the egg, and the stage of the ovotid is reached.

During the process of formation of the second polar body the spermatozoon is changing into the sperm nucleus (figs. 10, 12), in that its head becomes a rounded chromatin mass lying within a clear vacuole. Quite frequently there is polyspermy, but I have no evidence that such cases develop into embryos. When the second polar body is cut off we find the sperm nucleus in the form of a large spherical nucleus, with a nucleolus and a linin network; and the egg nucleus in a less advanced stage (fig. 13). Finally the two nuclei are seen, both in the rest stage (fig. 14). It is remarkable, and to my knowledge unique in the known cases of fertilization, that the two pronuclei are unequal in volume in the rest stage (fig. 14), as also in the prophases of the first cleavage (fig. 15); not a single case was found in which they were of equal volume. The smaller one appears to be the egg nucleus, because in most cases it is the one nearest the second polar body. Its smaller size is probably due to the fact that it is formed in less time than the sperm nucleus, and that the period is very short between its rest stage and the formation of the first cleavage spindle.

Fig. 15 shows the two pronuclei in the prophase of the first cleavage, the sperm nucleus being the one at the left hand; the nuclear membrane of each has disappeared at the point where there is an accumulation of finely structured cytoplasm, resembling the "archoplasm" of the similar stage in *Ascaris*. Each pronucleus contains a linin network and seven minute chromosomes; these are shown in the drawing much less distinctly than in the preparation, where the chromosomes are stained intensely blue and the linin very faintly. The first cleavage spindle (fig. 16) contains 14 chromosomes (only 12 seen in this figure), and the spindle, unlike the polar spindles, is pointed at each end; but neither at this stage nor at any of the later ones have I been able to determine the presence of centrosomes.

The double egg-membrane, which we saw had become separated from the surface of the egg, is still present and continues to include the embryo until the larval stage is a week old or more. It is a complete protection for the egg against any bacterial organisms.

In the matter of the cleavage I have spent much time in the endeavor to work out the cell-lineage, but have been unable to do so. The egg is spherical; the second polar body varies in its position with regard to the axis of the first cleavage spindle; the yolk appears to be rather uniformly distributed near the periphery. So there is no means of orientation of the uncleaved egg. The cleavage is total and adequal (figs. 17–21). The two blastomeres of the 2-cell stage are almost equal in volume, but one is always slightly larger than the other (fig. 17). In the 4-cell stage (fig. 18) there are two larger blastomeres and two smaller ones, but the difference is very slight. The axes of the spindles in the 2-cell stage may be parallel or not, and the cells may divide simultaneously or successively. In the 4-cell stage it is most frequent that a line connecting the two smaller blastomeres is perpendicular to one joining the two larger, in such a way that all four blastomeres do not lie in one plane; they apparently never arrange themselves in the T-shape so characteristic of *Ascaris*. Then the blastomeres are not spherical, but so moulded by mutual contact that it is exceedingly difficult to determine their relative volumes. Again, the blastomeres of the 4-cell stage may divide simultaneously or not. For these reasons I have found it impossible to work out the cell-lineage, without, however, wishing to imply that it may not be perfectly determinate.

A cœloblastula (fig. 20) is formed at an early cleavage, sometimes as early as six blastomeres, almost always at the 8-cell stage (fig. 19). At the 16-cell stage (fig. 21) the cleavage-cavity is large. On optical sections one pole of the blastula shows cells somewhat larger than those of the opposite pole.

While there are but a relatively small number of blastomeres, an invagination of the larger-celled wall of the blastula commences and leads to the establishment of a typical invagination gastrula (figs. 22–27). All the lining (mesentoderm) of the gastrocœl is not formed by an inpushing of the wall of the blastula, but it appears rather that only a small number of cells are invaginated, and that by the cell division of these the mesentoderm increases in amount. The orifice of the invagination, the blastopore is very narrow, and in a position that corresponds with the ventro-posterior end of the larva; and the end toward which the mesentoderm turns (figs. 23, 24, 26) is anterior; so we can now distinguish anterior and posterior, dorsal and ventral, right and left. The invaginated layer of cells is a mesentoderm, because from it comes both the mesenchym and the entoderm, as shown in figs. 25–27, where the mesenchym cells are shaded. From that area where the mesentoderm joins the ectoderm (the outer cell-layer) takes place a proliferation of cells of the mesentoderm, leading to the formation of loose cells and masses of cells, the mesenchym, lying within the cleavage-cavity. Fig. 23 shows just at the blastopore two particularly large blastomeres, which I first took to be mesenchym pole cells; but I found them in only one case, and do not believe there is a proliferation from a pair of pole cells, though the proliferation comes from the region near the blastopore. In no case was there any evidence of formation of the mesenchym from the side of the ectoderm; in these stages the mitotic spindles of the ectoderm cells lie always parallel to their free surface, never perpendicular to it, as would be necessarv for the formation of mesenchym. Particularly decisive in this matter are cases like that shown in fig. 25—mesentoderm cells dividing at right angles to the surface of the mesentoderm. This embryonic tissue is properly *mesenchym* and not *mesoderm*, since the cells do not arise as an epithelium or in compact masses, but separately; and cases like that of fig. 27, where they appear for a time to compose solid masses, are delusive and due to the difficulty of distinguishing the cells. At no stage up through the larva do these cellular elements become epithelia.

The remainder of the early development may be traced rapidly, and simply in order to clarify the organization of the larva. The blastopore closes early (*Bl.*, figs. 24–29), or remains as a very narrow opening. The entoderm grows forward and is largest at its apex (fig. 26); this portion of the entodermal canal will become the gland of the larva (figs. 28–30, *Gl.*). By cell division the few first-produced mesenchym cells form more numerous smaller elements within the cleavage-cavity, shown as the shaded cells in figs. 26–32. The next notable change is that at a point nearly opposite the blastopore (*Bl.*) the ectoderm thickens (fig. 28), becoming two to three cell-layers deep, whereas elsewhere it has become much thinner; at this stage also the anterior end of the entodermal canal is becoming constricted off from the remainder. In the next stage the thickened ectoderm mass commences to invaginate (fig. 29), this being the commencement of the proboscis; at the inner surface (that surface bordering on the cleavage-cavity) this invaginated ectoderm shows an annular proliferation (*Di.*, fig. 29), which is the perceptible beginning of the diaphragm of the larva. Now also the anterior portion of the entodermal canal is nearly cut off from the posterior, it is the immature condition of the larval gland (*Gl.*); sometimes, as in fig. 29, its anterior end is pointed and in contact with the ectoderm of the proboscis, and probably this pointed end represents the beginning of the tube duct of the gland; at least I have no further evidence as to the mode of development of the duct. Fig. 30 shows the ectodermal invagination further progressed, and the gland (*Gl.*) completely cut off from the intestine (*In.*); the mesoderm cells are numerous. In the stage of fig. 31 the embryo is bent upon itself, comma-shaped, since the growth is not of volume but of length, and the double egg-membrane prevents growth in a straight line. The embryo is bent in the region of the diaphragm (*Di.*). The diaphragm and the whole proboscis anterior to it is ectodermal, except for a few mesenchym cells (shaded) that have now become young muscle cells. Behind the diaphragm, all that region which may be termed the "trunk," we find entoderm composing the embryonic intestine (*In.*) and gland (*Gl.*), mesenchym (shaded), and a thin layer of ectoderm which, on the ventral surface near the diaphragm, is thickened (embryonic nervous epithelium). Fig. 32 is an immature larva with the armature of the proboscis developed.

During these stages the ectoderm of the surface of the body becomes very thin, and its component cells fewer in number (compare figs. 27 and 31); this is evidently due to a participation of the whole ectodermic layer in the proboscidial invagination. In the intestine also the nuclei move to the two ends (compare figs. 29 and 32), so that elsewhere the intestinal wall becomes very thin. The blastopore is completely closed, but its position still marked by the angle of junction of the ectoderm (hypodermis) and entoderm (intestine). The gland loses its central lumen.

2. The Larva (Plate L).

The larva is still included within the double egg-membrane, and is not noticeably larger in volume than the egg-cell, so that during all this time it can have taken no nourishment from without.

A transverse diaphragm (fig. 36, *Di.*), the margins of which are fused with the hypodermis (*Hy.*), separates the body into two regions an anterior proboscis and a posterior head-trunk. The diaphragm is with difficulty demonstrable, somewhat thicker than the hypodermis. and staining slightly with hæmatoxyline; on its anterior surface are a few nuclei, of which four central ones are notably conspicuous. It is traversed only by the duct (*Gl.d.*) of the gland (*Gl.*). It is owing to the presence of this diaphragm that movements of the proboscis cause no movements whatever of the fluid within the cavity (archicœl) of the head-trunk.

The proboscis is seen in evaginated condition in fig. 36, in contracted in fig. 37. The evagination is due to the contraction of longitudinal muscles (*Mus.*) lying beneath the body wall and reflected over the axial stalk of the proboscis. A contraction of these muscles evaginates the armature, partly perhaps by a direct pull, partly perhaps by producing a pressure upon the fluid contained within the cavity of the proboscis. A comparison of figs. 36 and 37 furnishes the best representation of the mechanics of this process. When the armature is evaginated the wall of the proboscis shows its annular folds very close together (fig. 36); when retracted, further apart and less numerous. What causes the retraction of the armature is difficult to decide, but it is perhaps due to the tension of certain long cells (*Fib.*) lying at the base of the axis.

The hypodermis of the outer wall of the proboscis is exceedingly thin from the diaphragm to the base of the third row of spicules (*Sp. 3*) of the armature, and contains not more than one or two nuclei. On its outer surface is a thicker homogeneous cuticula which does not extend over the armature region. Cuticula and hypodermis together compose a very thin outer wall which is thrown into folds, so that the larva appears to be superficially annulated. As well as I could determine these folds are temporary, and their number changes with movements of the proboscis, though the annulations are always present. There are no circular muscles to produce them. The hypodermis is thickened below the spicules and stilets which compose the armature, and shows one nucleus at the base of each stilet and each spicule; at the base of the stilets (*St.*, figs. 36, 37) the hypodermis becomes continuous with the axial ectodermic cell mass.

The armature, best studied in life or on individuals treated with caustic potash, shows three rows of spicules and three stilets (figs. 33–37). The first row of spicules (*Sp. 1*) is composed of a circle of six spicules, namely, a pair of dorso-lateral and a pair of dorso-ventral spicules, and a pair of lateral ones considerably larger than the others. The second row (*Sp. 2*) is made up of a circle of four spicules, a dorso-lateral and a ventro-lateral pair lying beneath the corresponding pairs of the first row; these are the smallest of all the spicules. The spicules of both of these rows are flattened, pointed cuticular plates, with slightly thickened margins. The third row of spicules (*Sp. 3*) is composed of a circle of needle-like spines, seven in number: one dorsal, one pair of dorso-lateral, one pair of dorso-ventral, and one pair of closely approximated ventral spicules. All these spicules project backward when the armature is evaginated. The three stilets point forward; one of them is ventral (*St.v.*, figs. 33–35), and the others dorso-lateral (*St.d.*). Each is a slender rod, with the proximal end widened, but with a depression on each side of the expansion (there shaped like a human red blood corpuscle); and with the distal end also expanded and bearing on its median surface a series of fine parallel ridges. The stilets surround a delicate canal, at the base of which opens the duct of the gland (*Gl.d.*). The whole armature is an exquisite device for the penetration of the tissues of the host, and for moving through them—the stilets penetrating, the spicules by their gripping pulling the larva forward. One does not know which to admire more, the perfection of the larva for occasioning torture or the beneficence of the Providence allowing such torment.

The axis of the proboscis is separated from the outer wall by a space, archicœl, and is composed of a cord of cells extending from the base of the stilets to the diaphragm (figs. 36, 37). That part of this cord nearest the stilets passes over continuously into the hypodermis, and is composed of a mass of cells without perceptible boundaries, containing a number of large nuclei. So far as I can determine these are simply undifferentiated ectodermic cells. The other end of the cord is made up of a number of long spindle-shaped cells (*Fib.*), attached at one end to the diaphragm; these may serve as elastic retractors of the evaginated armature.

The musculature of the proboscis is composed exclusively of longitudinal fibres (*Mus.* of figs. 36 and 37), the only faintly shaded structures in these drawings of the proboscis. These muscle cells are too minute for any determination of their finer structure; one can simply determine their long spindle shapes, the presence of nuclei in them, and the fact that they do not form a continuous layer, but are separated from one another though parallel. Their arrangement is shown in figs. 36 and 37.

All this region anterior to the diaphragm has been called by me the proboscis, because evidently the whole is simply an organ for penetration and locomotion in the host, even though it composes about one-half of the body. There is no entoderm in its constitution (except the duct of the gland body situated behind the diaphragm), and mesenchym only in the form of the musculature; all the remainder is ectodermic. It has no intestine, and no nervous system, unless, indeed, the large cells of the axis may be considered embryonic nerve cells, or some of the cells on the anterior surface of the diaphragm. It is not comparable with a head, nor is it to be regarded as a segment of equal morphological value with the head-trunk. The proof is yet wanting, but it will probably be found that this proboscis is the essential larval organ, disappearing more or less completely before the adult condition, and that the head-trunk is the persisting region.

The posterior head-trunk (fig. 36) is bent upon the proboscis, as the larva lies within the egg-membranes, but by the action of the longitudinal muscles the whole body may be straightened. Its hypodermis and cuticula is of the same structure as that of the posterior portion of the proboscis, except for the presence of two pairs of cuticular hooks (*H.*), placed right and left on each side of the posterior end, and for the presence of a thickening of the hypodermis. This thickening (*Nv.*) lies ventral just behind the diaphragm, and shows a double row of large nuclei, one row to the right and the other to the left of the median line, and each row with four to five nuclei. This thickening is to be regarded as the nervous system of the larva. It is an ectodermal thickening of definite form in every larva; and its ventral position corresponds with the position of the nerve cord of the adult. There are no other parts of the larva which can be considered nervous, unless the large hypodermal cells composing the axis of the proboscis have that function—against which assumption a number of objections might be urged. This thickening is then a double row of neuroblasts, recognizable only on carefully stained preparations; nerve fibres cannot be determined.

The longitudinal muscle-cells (*Mus.*) lie just beneath the hypodermis, and terminate anteriorly against the diaphragm. They have the same appearance as those of the proboscis, but are more difficult to perceive, particularly those of the dorsal side. Here also there is no trace of circular muscles.

In the archicœl, at the anterior end of the head-trunk, is placed a large gland (*Gl.*), which, as we have seen, arose as an abstriction from the entodermal canal. By a long duct (fig. 36, *Gl.d.*) it is connected with the exterior at the base of the stilets (*St.*) of the proboscis. This gland lies free within the archicœl, and contains about eight large nuclei; cell boundaries are barely distinguishable, and the cytoplasm is dense and stains uniformly. The long, convoluted duct can usually be found only in life, when it appears as a perfectly clear, convoluted line. On a few preparations, however, its portion within the gland body was deeply stained by hæmatoxyline. It arises near the posterior end of the gland body, extends through the length of the latter, then piercing the diaphragm extends forward through the archicœl of the proboscis, *i.e.*, on the surface of the axial stalk. Whether this extremely delicate canal is intercellular or intracellular in the gland body, I could not ascertain; if it were intercellular it would represent a portion of the gastrocœlic cavity. As to the function of this gland, the position of its external aperture at the base of the stilets would point to its being a poison gland. There is no good reason for judging it to be an excretory organ; and indeed we shall see that what are probably excretory masses become stored up within the cavity of the intestine.

The intestine (*In.*, fig. 36) of the larva is a closed tube, terminating blindly at its anterior end, and at its posterior connecting with the cuticula of the body wall without external opening; this latter point corresponds with the blastopore (*Bl.*). This posterior end of the intestine appears as a narrow, solid stalk, with two or four nuclei apposed to its surface. The wall of the remainder of the intestine is very thin, except at the opposite ends where it is thickened and embraces several large nuclei. Within the intestinal lumen are always present usually two, sometimes more, large globules (*Glo.*) of a pale brownish color and viscid consistency. These appear first when the larva is not quite mature as a thinner fluid, but subsequently accumulate as homogeneous, rounded globules. These cannot represent food substance, for the blastopore is closed and the intestine has no connection with the exterior. It is then probable they represent products of metabolic waste, in which case the intestine of the larva would serve as an excretory reservoir.

Within the archicœl, just at the posterior end of the head-trunk, is a mass of a few small cells (*Mus.*), evidently mesenchym cells that have not become specialized; they are embryonic, and may be germ cells.

Finally, the body-cavity of the larva is an archicœl, without floating cells and apparently without connective tissue. There are no mesodermal epithelia (mesothelia); and the only transverse septum of the body, the diaphragm, is ectodermic.

3. Previous Work.

I have not seen the papers of Grube (1849) and Meissner (1856), nor yet the last paper on the early development, one in Russian by Tretiakow (1901).

Leidy (1850, 1870) gives a very superficial account, with no figures, of the formation of the larva; he noted the proboscis invagination, and the spicules ("filaments") of the armature.

Villot (1874) was the first to give an extended account of the development of the larva. He described the formation of the egg-string ("nidamentum"), and the appearance of the egg-envelopes; and describes the polar bodies as being very variable in number, form and volume. He found the segmentation to be total and equal and leads to a "germe" formed of two concentric spheres (the process of which was not determined), ectoderm and entoderm respectively. He noted the ectodermal invagination at the anterior end of the embryo and interpreted it to be the beginning of the "head" (equivalent to my term "proboscis"). His fig. 49 is the most detailed sketch yet given of the structure of the larva. The whole armature of the proboscis is described very accurately. But he describes an anterior mouth communicating by an œsophagus with a posterior intestine, and the latter by an open anus with the exterior—failing to see the diaphragm, and mistaking for an œsophagus the solid axial cord of the proboscis. He saw correctly the gland and its duct, but interpreted it as an excretory organ. The musculature is noted, and the body-cavity described as filled with cells. His second paper (1891) is mainly a criticism of the work of Camerano.

Camerano (1889) studied the development not quite to the point of the larval stage, figures the chromosomes and certain details in the formation of the polar bodies, which he finds to be two in number. The earliest penetration of the spermatozoon he did not see, but describes a succession of stages of the two pronuclei, and figures them as of unequal size. The early cleavage he finds to be quite variable in different eggs, though holoblastic. In regard to the process of gastrulation his results are entirely different from mine. He finds the embryo at the end of the cleavage to be a flattened plate, not a sphere, a disk composed of two layers of cells—a sterroblastula. This plate becomes quadrangular instead of circular in outline; then an invagination takes place at one end of the plate, which he interprets as a prostoma, and concludes the sterroblastula to have become a cœlogastrula. I can only interpret Camerano's results as follows: First, that the strong fixatives employed produced flattening of the embryos, which I have frequently observed after fixation with acetic acid or strong alcohol; second, that he did not see the gastrula invagination at all (which would take place at the stage of his figs. 43 to 45), and mistook the ectodermal proboscidial invagination for the gastrula cavity.

Villot was in error in describing the true larva as the "état embryonnaire" or "première forme larvaire," and the immature worm found as a parasite in insects the "état larvaire proprement dit" or "deuxième forme larvaire." Camerano and Vejdovský (1894) are quite right in insisting, in opposition to Villot and von Linstow, that the organism as it leaves the egg-membranes is the larva, and that there is no second larval stage. That stage is the larva which is equipped with a temporary larval organ, the proboscis ; all later stages, as far as they are known, are but progressions toward the adult condition.

4. Conclusions.

The opinion I had previously expressed (1903) from a study of the adult anatomy of *Paragordius* was as follows: "With the Nematoda the Gordiacea have in common only one important structure, the tubular genitalia and their opening into the cloaca. With the Annelida they agree in the structure and innervation of the musculature, and in having dorso-ventral mesenteries which divide body-cavities bounded by cell-layers. But there is good reason for doubting the homologies of these mesenteries and body-cavities in these two groups of animals. They differ from the Annelida in showing no trace at all of true metamerism (either in the nervous system, the body-cavities, the genitalia or the body wall); in the absence of a cerebral ganglion, of a vascular system, of setal sacks or extremities; and very markedly in the structure of the genitalia. In view of these facts the Gordiacea cannot be regarded as even highly degenerate Annelida, as Vejdovský has done, nor yet as modified Nematoda, the view of most of the writers, but must rather be considered a group of isolated position, as Grenacher, von Siebold and Villot have regarded them."

The present study of the early development confirms this view, but shows that there is considerable correspondence of the cleavage and gastrulation processes with those of the Nematoda. The middle germ-layer is not a connected mesoderm, but a mesenchym of disconnected cells, arising from the mesentoderm close to the blastopore.

The larva has two marked peculiarities. First, by a transverse septum, the diaphragm, the body is partitioned into an anterior proboscis and a posterior head-trunk. This proboscis is entirely ectodermal except for mesenchymatous muscles, and lacks any nervous system, excretory system, and any portion of an intestine. Though it composes half the body, it is probably a purely larval organ that takes no part in the adult structure; the diaphragm marks the future boundary of the adult head, and the proboscis is precephalic. A support for this view, apart from good anatomical considerations, is to be found in the work of Vejdovský (1894), particularly his fig. 17; this represents an outline of the head of an immature individual, showing at the tip of the definitive head the still persisting proboscis with a transverse septum. The future head and trunk proper then lie behind the proboscis of the larva, and this head-trunk becomes the complete body of the adult; this we can say certainly, even though the early post-larval development is entirely unknown.

This head-trunk has a wall like the proboscis, a hypodermis with cuticula, and longitudinal mesenchymatous muscle fibres. There is no cœlom, no peritoneum or mesenteries. The nervous system is simply a ventral thickening of the hypodermis. In the head-trunk is found the second striking peculiarity of the *Paragordius* larva—a gland connecting by a long duct with the anterior end of the proboscis. This gland is entodermal, arising as an abstriction from the anterior end of the entodermal tube. In point of origin it resembles an enterocœl, a mesoderm sack, and its early lumen is a portion of the gastrocœl. But there the resemblance ceases, for it becomes a gland, evidently secretory and not excretory, which later develops a duct to the exterior. Its origin is unpaired. The only forms which show a similar unpaired enterocœl arising from the anterior end of the entoderm tube are the Holothurians, and there an outgrowth of a portion (hydrocœl) of the hydro-enterocœl connects it, in the form of the stone canal, with the exterior. But it would be a rash comparison, in no way justified, between a Gordiacean and a Holothurian. This gland probably continues to a late stage of the development under the form of the "braune Drüse" of *Gordius,* described by Vejdovsky, but is not homologous with the problematical "supraintestinal organ" that I found in the adult of *Paragordius.*

When we consider this ectodermal proboscis, the ectodermic diaphragm and the enterocœlic gland; the absence of any cœlomic cavities, ciliary wreaths and excretory organs; the lack of a mouth and the mesenchymatous musculature, we must conclude this union of characters to make the Gordiacean larva unique, not closely comparable with any other larva.

If my interpretation of the larva is correct, then the early post-larval development, which has yet to be studied, should show the mouth arising in the plane of the diaphragm, probably as an ectodermic stomodæum; the dorsal commissure of the brain developing in the same region; and the reproductive organs arising from the small mass of mesenchym cells at the posterior end. But the point of most importance that remains to be investigated is the first origin of that tissue so peculiar to the Gordiacea, the "parenchym," and its arrangement into layers. Not until that is done can the structure of the adult be adequately interpreted.

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EXPLANATION OF PLATES XLIX AND L.

The following abbreviations have been employed in the figures:

Bl., blastopore.
Cut., cuticula.
Di., diaphragm.
Fib., fibre cells.
Gl., gland.
Gl.d., gland duct.
Glo., globules within intestine.
H., cuticular hooks.
Hy., hypodermis.
In., intestine.
Mes., mesenchym cells.
Mus., musculature.
Nv., nervous thickening of hypodermis.
Sp. 1–Sp. 3, spicules of rows 1–3.
St.d., dorsal stilets.
St.v., ventral stilet.

All the figures, except 33–35, are camera lucida drawings at a magnification of 1312 diameters.

PLATE XLIX, Fig. 1.—Ovocyte from lateral diverticulum of ovary, at end of the growth period; the yolk globules shaded.

Figs. 2–5.—Nuclei in successive prophases preceding the formation of the first polar body.

Fig. 6.—Portion of an egg near the proximal end of the cloaca, showing the first polar spindle and the entering spermatozoon.

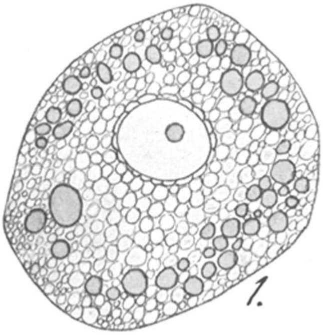
Fig. 7.—Pole view of the first polar spindle.

Fig. 8.—Outline of the whole egg, from a posterior end of the cloaca, showing the two egg-membranes, first polar spindle and spermatozoon.

Fig. 9.—Portion of the periphery of an egg, showing first polar body formed.

Fig. 10.—Outline of egg, showing second polar spindle, sperm nucleus and, separated from the surface of the egg, the two membranes.

Fig. 11.—Pole view of second polar spindle.
Fig. 12.—Outline of egg, sperm nucleus and expulsion of second polar body.
Figs. 13, 14.—Outline of eggs, second polar body on surface, egg nucleus at right, sperm nucleus at left in both figures.
Fig. 15.—Prophase of first cleavage, seven chromosomes in each nucleus; sperm nucleus at left, egg nucleus at right; yolk globules shaded.
Fig. 16.—First cleavage spindle and second polar body.
Figs. 17–19.—2-cell, 4-cell and 8-cell stages.
Fig 20.—Optical section of a blastula.
Fig. 21.—16-cell stage.
Figs. 22–24.—Successive gastrula stages, optical median sections.
Figs. 25–27.—Optical median sections of later gastrulæ, showing the early formation of mesenchym cells (shaded).
Fig. 28.—Optical median section at stage of ectodermal thickening; mesenchym shaded.
Fig. 29.—Optical median section at stage of beginning ectodermal invagination; mesenchym shaded.
Figs. 30–32.—Similar views of successive later stages.
Figs. 33–35.—Freehand drawings to show the armature of the proboscis in extended position. 33, slightly oblique ventral view; 34, view from the right side; 35, slightly oblique dorsal view.
Plate L, Fig. 36.—Freehand drawing, on a much larger scale than on the preceding plate, of a larva from the right side, with the proboscis fully extended.
Fig. 37.—Similar drawing of the proboscis in invaginated condition.



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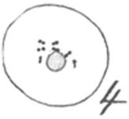

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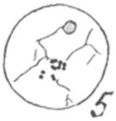

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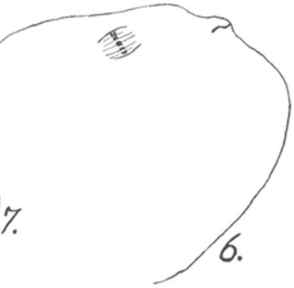

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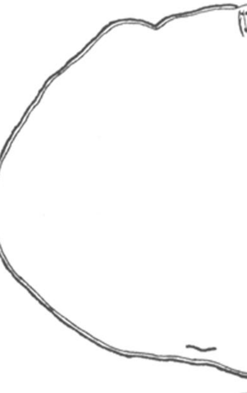

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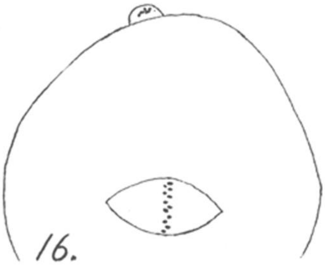

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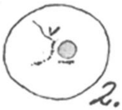

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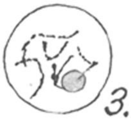

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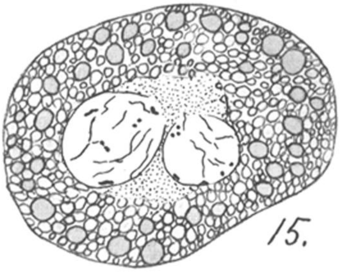

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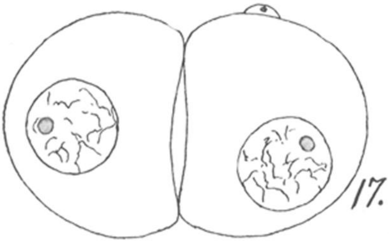

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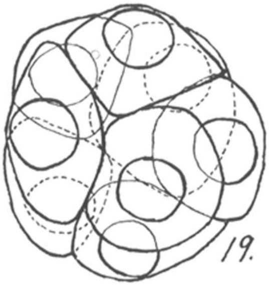

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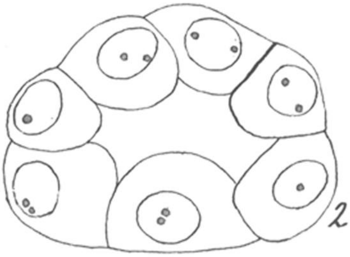

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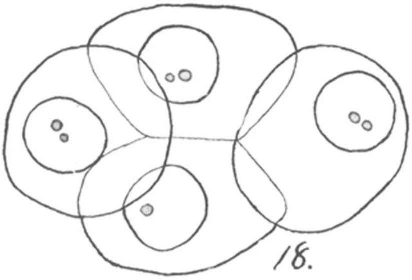

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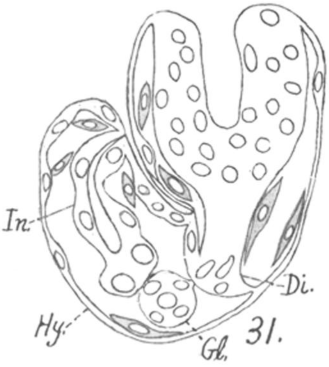

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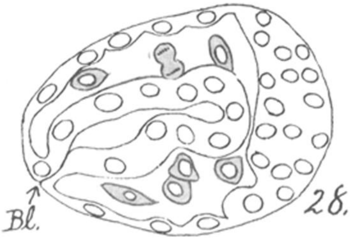

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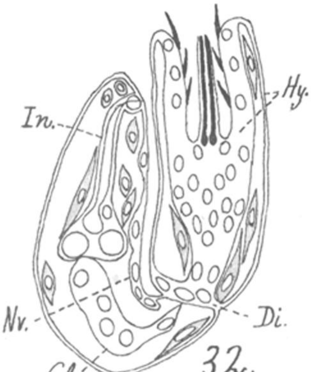

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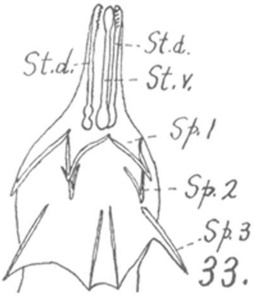

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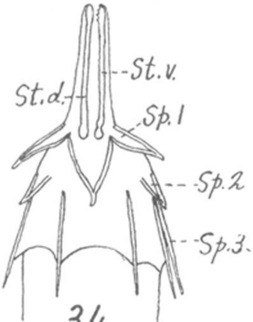

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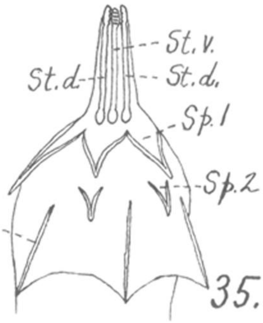

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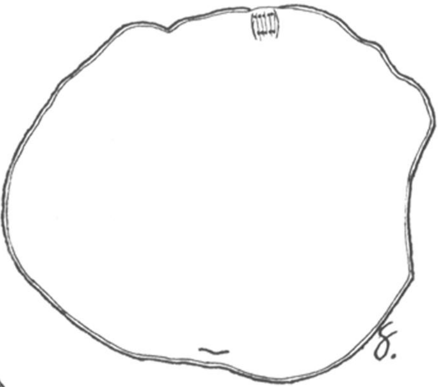
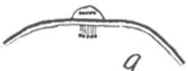
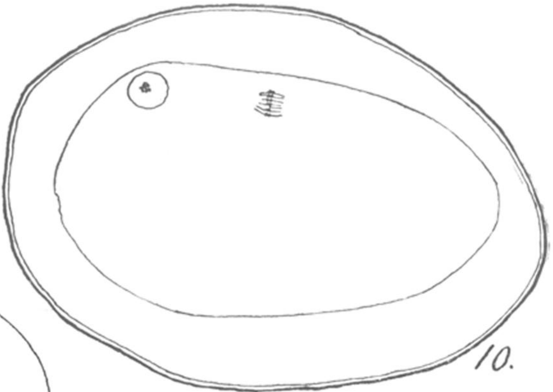
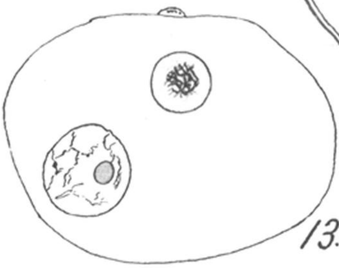
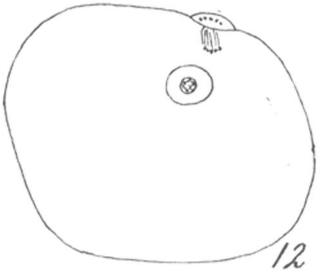
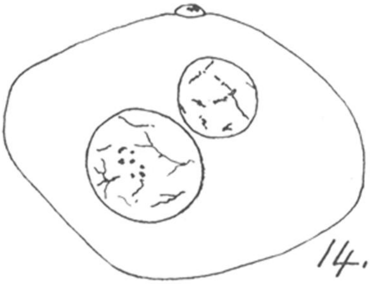


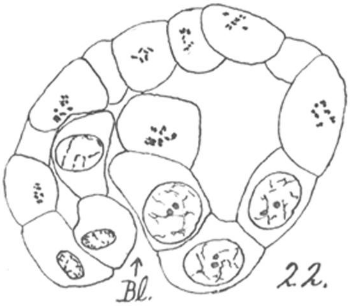


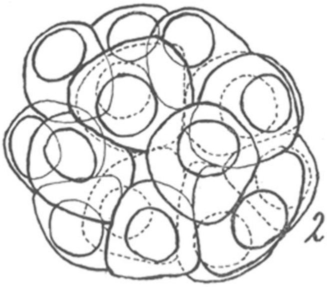


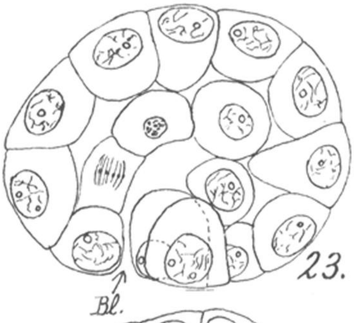


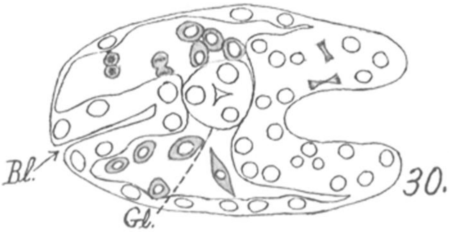


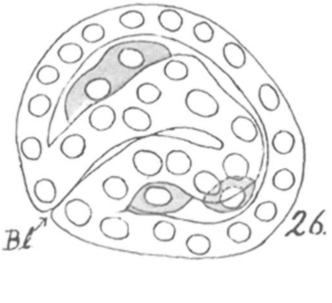


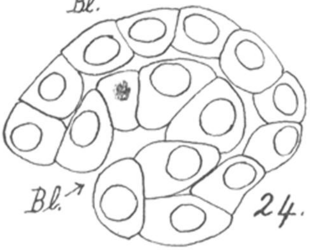


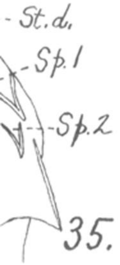


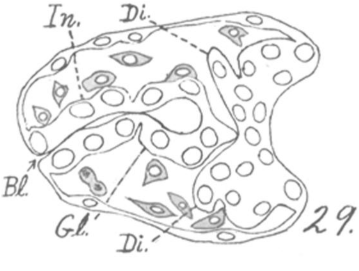


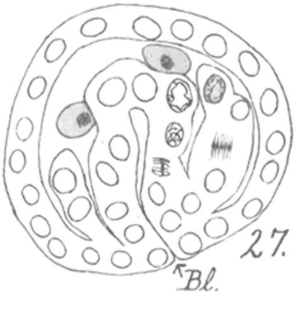


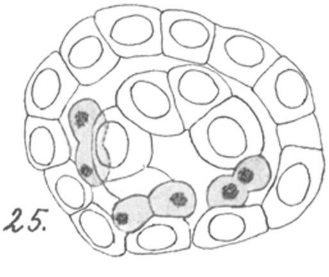




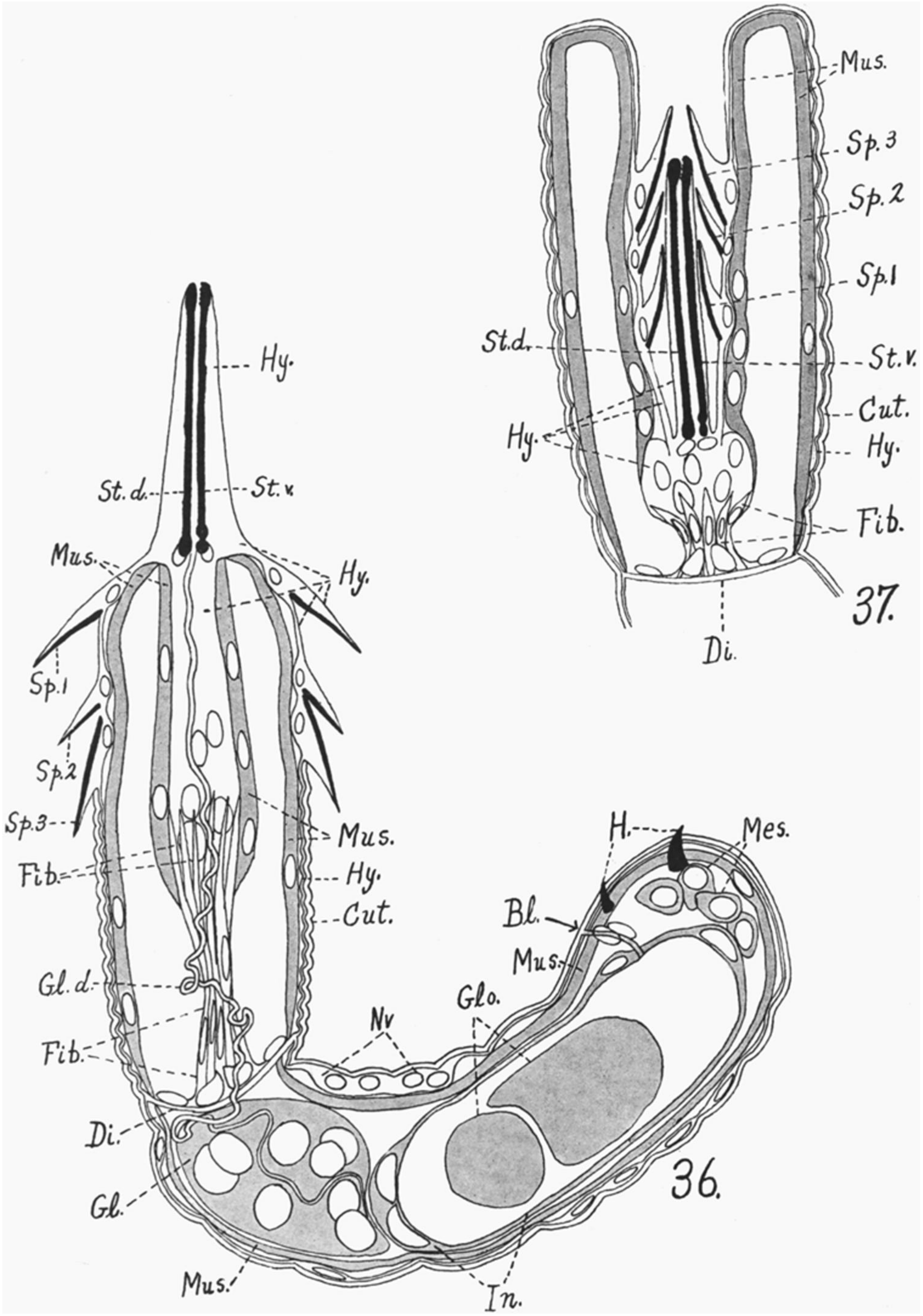








Y. LARVA OF PARAGORDIUS.



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